Universidade de Lisboa
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Factors Influencing Primary Stability of Dental Implants Assessed with Resonance Frequency Analysis: An *In Vitro* Study

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Dissertation supervised by
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Aos meus pais e ao meu irmão,

Que são tudo para mim.

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Abbreviates

PS	Primary Stability
RFA	Resonance Frequency Analysis
PT	Periotest
IL	Immediate Loading
IDL	Immediate-delayed Loading
DL	Delayed Loading
SS	Secondary Stability
ISQ	Implant Stability Quotient
CT	Computerized Tomography
IT	Insertion Torque
PTV	Periotest Values
MPP	Manufacturer Preparation Protocol
APP	Altered Preparation Protocol

Abstract

Purpose: To evaluate the influence of two different surgical preparation protocols in the primary stability (PS) of implants; To evaluate if the probe's tip position and sterilization process of the SmartPeg influences the measurement of implant PS, obtained through resonance frequency analysis.

Materials and Methods: Twenty implants ImplanteDouble® (Conexão, Rubeaspharma, Porto, Portugal) with 4.0x8.5 mm were placed in cow ribs. In groups A, B, C and D the implant site preparation was made according to the manufacturer recommendations, and implants were placed with a 60 Ncm insertion torque. For groups E, F, G and H the initial preparation was the same of the manufacturer's protocol groups, but with an additional drill. Implants in those groups were placed with a 40 Ncm insertion torque. Implant PS values were obtained trough Osstell® Mentor (Osstell AB, Gothenburg, Sweden). In groups A, B, E and F six measurements were taken for each implant, using a new SmartPeg. Three of them were obtained positioning the probe parallel to the SmartPeg and other three positioning it perpendicularly. The same protocol was used for implants in groups C, D, G and H. However, in those groups a re-used SmartPeg was employed instead of a new one. For each group the sample size were 10 implants.

Results: There were statistical significant differences ($p < 0.05$) between the ISQ values of implants placed in accordance to the manufacturer recommendations and following an altered preparation protocol, with implants in Groups A, B, C and D presenting higher values of PS. No significant differences were observed between ISQ values recorded with different probe positions and using a new or a 1 cycle of sterilization subjected SmartPeg.

Conclusions: The surgical preparation protocol influences PS. Different positions of probe's tip and sterilization of the SmartPeg, do not affect PS measurements.

Keywords: Dental Implants, Primary Stability, Surgical Technique, Resonance Frequency Analysis.

Resumo

Em 1969, Branemark descobriu que era possível estabelecer uma ligação direta entre o osso alveolar e os implantes. Desde então múltiplos estudos têm sido desenvolvidos com o propósito de maximizar as taxas de sucesso da terapia implantar, considerando um vasto conjunto de situações clínicas possíveis, tendo-se observado, ao longo dos anos e em resultado dos avanços alcançados, um aumento crescente nas taxas de sucesso dos implantes dentários.

A estabilidade primária (EP) pode ser definida como a ausência de mobilidade do implante no osso imediatamente após a sua colocação, e constitui o parâmetro que permite prever o sucesso clínico dos implantes. Assim sendo, assume particular importância a determinação precisa do seu valor. A análise da frequência de ressonância consiste num dos métodos que permite justamente medir a EP dos implantes. Concretamente, através da medição da rigidez do sistema transdutor-implante-osso, expressa em unidades ISQ (Implant Stability Quotient). Nas versões mais recentes deste sistema de análise (Osstell® Integration Diagnostic AB, Sweden), o transdutor consiste num pilar metálico magnetizado designado por "SmartPeg" que é enroscado ao implante para realizar as respetivas medições.

A análise da frequência de ressonância foi inicialmente descrita por Meredith e colaboradores em 1994. Dois anos depois, surgiram os primeiros estudos utilizando esta técnica para obter os valores de EP dos implantes. Presentemente, a mesma é considerada como altamente fiável e reprodutível, em comparação com os outros métodos existentes.

Existem vários fatores que influenciam os valores de ISQ. Nomeadamente, a densidade óssea do leito implantar, o comprimento e diâmetro do implante, e a rigidez com que o SmartPeg é colocado. Adicionalmente, a posição da sonda do Osstell, durante a aquisição do ISQ, poderá também apresentar influência nas medições dos valores de EP. Contudo, relativamente a este possível fator de influência, os estudos disponíveis na literatura são contraditórios. No que diz respeito ao SmartPeg, o fabricante adverte que se trata de uma peça de utilização única. Justificando com o facto de que a remontagem, bem como as condições extremas às quais o SmartPeg é submetido durante o processo de esterilização danificam o mesmo. Desta forma, quando reutilizado o SmartPeg, a precisão dos valores de ISQ obtidos não poderá ser assegurada. Assim sendo, existe a possibilidade de que, quer o posicionamento da sonda do Osstell, quer a esterilização do SmartPeg possam influenciar as medições da EP,

conduzindo assim a interpretações erradas que poderão comprometer o sucesso da terapia implantar.

Vários fatores influenciam a estabilidade primária dos implantes: os relacionados com o osso em torno do implante (quantidade e qualidade do mesmo); os que dizem respeito ao próprio implante (design e morfologia); e, finalmente, a técnica cirúrgica com a qual os implantes são colocados. No que diz respeito a esta última é importante referir que não só afeta a EP como também a estabilidade secundária, a qual, por sua vez depende essencialmente dos processos de modelação e remodelação óssea, sendo que quanto maior for a quantidade de remodelação óssea, mais tempo será necessário para que o implante adquira estabilidade secundária. Ou seja, a técnica cirúrgica é extremamente importante na obtenção de uma forte interface osso-implante.

Em localizações com osso de elevada densidade, pode suceder, que durante a colocação do implante, o mesmo não consiga atingir a profundidade total no leito implantar. Perante tal situação, o clínico poderá aumentar o torque de inserção do implante ou alargar o leito implantar. É sabido que níveis elevados de compressão óssea, podem resultar em taxas elevadas de reabsorção óssea (devido à quantidade de necrose e de remodelação óssea que ocorrem). No entanto, os efeitos de elevados valores de torque de inserção permanecem ainda por esclarecer. Desta forma parece ser mais seguro alargar o leito implantar. Porém, tal poderá ter consequências negativas na EP dos implantes dentários.

Objetivos: Avaliar a influência de dois protocolos cirúrgicos diferentes na estabilidade primária dos implantes. Adicionalmente, avaliar se a posição da sonda do Osstell (perpendicular versus paralela) e a esterilização do SmartPeg influenciam as medições de EP obtidas através da análise da frequência de ressonância.

Materiais e Métodos: Foram colocados 20 implantes ImplanteDouble® (Conexão, Rubeaspharma, Porto, Portugal) de 4,0x8,5 mm em costelas de vaca. Nos grupos A, B, C e D a preparação do leito implantar foi realizada de acordo com as recomendações do fabricante para osso de elevada densidade, e os implantes colocados com um torque de inserção de 60 Ncm. Nos grupos E, F, G e H (protocolo de preparação alterado), a preparação inicial foi igual à realizada nos grupos do protocolo do fabricante, recorrendo-se, neste caso, à utilização de uma broca adicional com o intuito de alargar o leito implantar. Nestes últimos grupos os implantes foram colocados usando um torque de inserção de 40 Ncm.

Os valores de estabilidade primária foram obtidos através do Osstell® Mentor (Osstell AB, Gothenburg, Sweden). Nos grupos A, B, E e F foram realizadas seis medições em cada implante, usando um SmartPeg novo. Três delas foram obtidas posicionando a sonda do Osstell perpendicular ao SmartPeg e outras três posicionando-a paralelamente. O valor final de ISQ para cada implante foi obtido realizando a média das três medições. O mesmo protocolo de medição da EP foi usado para os implantes dos grupos C, D, G e H. No entanto, nestes grupos foi usado um SmartPeg já submetido a 1 ciclo de esterilização. A amostra para cada um dos grupos foi de 10 implantes.

A análise estatística dos resultados foi feita usando o teste não paramétrico de Mann-Whitney após se ter verificado que a amostra não seguia uma distribuição normal. Os testes de Kolmogorov-Smirnov e Shapiro-Wilk foram usados para avaliar se os resultados seguiam uma distribuição normal. O nível de significância estabelecido foi de 5%.

Resultados: Existem diferenças estatisticamente significativas ($p < 0.05$) entre os valores de ISQ dos implantes colocados de acordo com o protocolo cirúrgico recomendado pelo fabricante e os implantes colocados segundo o protocolo cirúrgico alterado. Os implantes dos grupos A, B, C, e D (protocolo do fabricante) apresentaram valores de EP superiores. Não foram observadas diferenças estatisticamente significativas entre os valores de ISQ obtidos através de diferentes posições da sonda do Osstell e usando SmartPegs novos ou submetidos a 1 ciclo de esterilização.

Conclusão: Tendo em conta as limitações do presente estudo *in vitro*, os resultados sugerem que, em osso de elevada densidade, o protocolo cirúrgico de colocação de implantes influencia a estabilidade primária dos mesmos. Adicionalmente, sugerem que ambos - posicionamento da sonda do Osstell (perpendicular versus paralela) e esterilização do SmartPeg - não influenciam as medições de EP obtidas através da análise de frequência de ressonância.

Estudos futuros poderão ser realizados para investigar o número de ciclos de esterilização a que o SmartPeg tem que ser submetido para que sejam observadas diferenças estatisticamente significativas nos valores de ISQ.

Palavras-Chave: Implantes Dentários, Estabilidade Primária, Análise da Frequência de Ressonância, Técnica Cirúrgica

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1. Literature Review and Clinical Relevance

1.1 Introduction

Due to the increase of success rates of dental implants over the years, and considering the great solution that implants became both in functional and esthetical terms, the interest of study this subject continues growing uninterruptedly. In 1969, Brånemark discovered that direct contact between bone and implant surface was possible (Brånemark et al., 1969; Brånemark, 1983). Since then multiple studies were performed with the same ultimate goal: to investigate how to magnify the success of dental implants, not only when the host conditions are ideal (adequate bone quality and quantity, and an overall good health), but also when there are inadequate bone conditions or some medical disorders that affect implant therapy.

Primary stability (PS) is the parameter that can predict the long-term clinical success of dental implants (Rabel et al., 2007). PS is defined as the absence of mobility of the implant in the bone bed after its placement (Shadid et al., 2014), Therefore, given the significance of PS on the dental implants prognosis, it is extremely important to determine its values during different stages of treatment. Over the years, several techniques have emerged for this purpose. Those techniques can be either invasive or non-invasive (Park et al., 2011; Quesada-García et al., 2009). As invasive techniques, we have, for instance, Histomorphometric Evaluation and Removal Torque (Park et al., 2011). On the other hand, Resonance Frequency Analysis (RFA) and Periotest (PT) are two examples of non-invasive techniques (Quesada-García et al., 2009). Some of these techniques have been continuously improved over time, since determination of PS should be used, according with some authors (Winter et al., 2010), as a basis for the selection of specific loading protocols. Other authors go further, stating that the success of particular loading implant's techniques, such as immediate and early loading, is dependent on the ability of the clinician to determine the degree of primary implant stability and changes in stability along with bone modeling and remodeling (Sennerby & Meredith, 2008).

Concerning RFA, it allows to quantitatively and qualitatively analyze the stability of various types of implants and examine their behavior under different bone and loading conditions, thus presenting great benefits to implant therapy (Quesada-García et al., 2009).

1.2 Osseointegration

About four decades ago, Brånemark et al. proved that direct contact between bone and titanium implant surface was possible, defining osseointegration as "the direct, structural and functional contact between living bone and the surface of a functionally loaded implant" (Brånemark et al., 1985). Ten years later, Schroeder et al. defined this bone-implant union as a “functional ankylosis”, stating that new bone formation in direct contact with implant surface will occur as long as the installation of the implant is atraumatical (Schroeder et al., 1981). Currently, osseointegration is accepted as a histological term symbolizing direct bone apposition on the implant surface with no interposition of soft tissue (Digholkar et al., 2015).

Lioubavina-Hack and colleagues conducted a study in male rats in which they concluded that primary implant stability is a prerequisite for successful osseointegration. Moreover, primary instability of implants will result in fibrous encapsulation of the implants (Lioubavina-Hack et al., 2005).

The clinical definition of implant osseointegration comprises the level of stable marginal bone around implant fixture and absence of mobility (Digholkar et al., 2015). Zarb & Albrektsson described osseointegration as a process in which a clinical asymptomatic, stable and rigid fixation occurs between alloplastic materials (dental implants) and bone tissue during functional loads (Zarb & Albrektsson, 1991). Therefore, to achieve osseointegration of dental implants, certain biological and biomechanical requirements must be met. One of the most important is the absence of micro-movements during the stage of osseous cicatrization (Herrero-Climent et al., 2012). It is known that, micro-motions higher than the threshold of 50 to 100 μm can lead to the formation of fibrous tissue and induce bone resorption at the bone-to-implant interface (Javed et al., 2013; Barikani et al., 2013). These may primarily be responsible for failure of osseointegration and ultimately, for implant loss (Javed et al., 2013).

Esposito et al. published a study about implant failure, stating that many different biological factors are contributing to the failure of implants osseointegration. They concluded that surgical trauma and anatomical conditions are the most critical factors for primary implant loss. On the other hand, jawbone quality, volume, and overload are major determinants for late implant failures. Yet, the impact of these factors on implant failure rate depends on the implant design and surface characteristics and can be influenced by the surgical technique (Esposito et al., 1998).

1.3 Loading Protocols

Several options could be used in order to load the implant: immediate loading (IL), immediate-delayed loading (IDL), and delayed loading (DL) (Meredith, 2008; Sennerby & Meredith, 2008).

In the IL protocol the prosthesis is directly connected to the implant earlier than 1 week after implant placement (Gallucci et al., 2014). Therefore, IL's success depends largely on the achievement of PS upon implant insertion and the absence of micro-movements during the healing period (Herrero-Climent et al., 2013). Several studies included in a recent Cochrane Systematic Review about different loading protocols concluded that poor implant PS is an exclusion criteria for IL (Esposito et al., 2013; Herrero-Climent et al., 2013).

In IDL approaches, implants may remain unloaded for a short period of time, between 1 week and 2 months, whereas DL, also known as conventional implant loading, corresponds to an unloaded period of at least 2 months, after which the prosthetic structure is placed (Esposito et al., 2013; Gallucci et al., 2014).

Over time, studies comparing these 3 different loading approaches have been published. Meloni et al. conducted a study where 40 implants were bilaterally installed on the posterior region of the mandible. On one side the implant was loaded within 24h after its placement, whereas on the opposite side the crown was provided only 4 to 5 months after surgery (Meloni et al., 2012). Den Hartog and colleagues compared the outcome of IL with DL for single implants in the maxillary aesthetic zone (N=62) (den Hartog et al., 2011). In both studies, the authors concluded that clinical outcomes (implant survival, complications, radiographic marginal bone-level changes and soft tissue aspects) of immediate *vs* delayed loading are comparable (den Hartog et al., 2011; Meloni et al., 2012). Other studies compared IDL with DL and reached the same conclusions (Payne et al., 2002; Tawse-Smith et al., 2002). Cannizzaro et al. evaluated the efficacy of IL vs IDL (6 weeks) on sixty patients. They concluded that there is no apparent advantage on waiting 6 weeks to load the implants (Cannizzaro et al., 2008). Other study, by Merli et al., concluded that the null hypothesis of no difference in failure rates, complications and bone level between implants that were loaded immediately or early cannot be rejected (Merli et al., 2012).

Esposito *et al*., in a systematic review about loading approaches, concluded that there was no convincing evidence of a clinically important difference in prosthesis

failure, implant failure, or even bone loss, associated with different loading protocols of dental implants (Esposito et al., 2013). However, in contrast with the other two protocols, DL can lead to predictable results in all clinical situations. For that reason, it is highly recommended in specific conditions, such as, poor PS, significant bone augmentation, implants of reduced dimensions, and compromised host conditions.

1.4 Implant Stability

Implant stability can be differentiated in two categories: PS and secondary stability (SS). PS has a major impact on the long-term success of dental implants, corresponding to the stability of the implant immediately after its insertion on the alveolar bone (Rabel et al., 2007).

PS mostly comes from mechanical engagement of the implant with cortical bone (Javed et al., 2013; Shadid et al., 2014; Digholkar et al., 2015), specifically, the result of compressed bone holding the implant tightly in place. For that reason, it is considered a mechanical stability (Digholkar et al., 2015). Furthermore, PS can be defined as the absence of clinical mobility. Ivanoff and colleagues investigated the influence of PS in a rabbit study. They concluded that high PS reduces the risk of micromotion and adverse tissue responses, such as fibrous tissue formation at the bone-implant interface, during healing and loading (Ivanoff et al., 1996). Following that, determination of PS has also been used as an indicator for future osseointegration and as a basis for the selection of specific loading protocols (Winter et al., 2010). In fact, Friberg and colleagues reported an implant failure rate of 32% for those implants that showed inadequate initial stability (Friberg et al., 1991).

In a clinical study of 12 weeks, Markovic et al. investigated strategies for optimization of implant stability in the low-density bone. The authors found significant decreases in implant stability in all study groups during the first weeks of the follow-up period. This corresponds to the transition from primary mechanical stability provided by the old bone, to biologic stability provided by the newly formed bone. During this period, osteoclasts remove the existing bone, and the amount of new bone formed are still insufficient to provide SS. In this way, after the fourth postoperative week, a tendency toward increased stability would be expected in all study groups, as a result of new bone formation (Markovic et al., 2011).

Unlike PS, secondary stability is considered a biological stability (Digholkar et

al., 2015) because of the biologic events that takes place during this stage at implant-bone interface (Shadid et al., 2014). It refers to the stability that implant acquires when bone formation occurs in direct contact with the implant surface, and that is determined by the process of osseointegration itself (Herrero-Climent et al., 2012). SS depends on bone modeling and remodeling at the implant-bone interface and is influenced by the implant characteristics (such as surface), surgical technique, wound-healing time, and also by the PS (Shadid et al., 2014; Digholkar et al., 2015).

Data published in the literature suggest that PS leads to predictable SS, which has shown to increase four weeks after implant placement. During those four weeks in the healing process, the lowest implant stability is expected. Clearly, the healing process will be affected by the bone morphology, more precisely, trabecular pattern and density, and stage of maturation (Sim & Lang, 2009). Meanwhile, as a result of osseointegration, initial mechanical stability (PS) is being replaced by biological stability (SS) (Mall et al., 2011; Sim & Lang, 2009).

Additionally, it is important to understand the factors that influence PS. They are several: those related to the bone surrounding the implant (quantity and quality), those related to the implant itself (design/morphology) and finally, the surgical technique (Rabel et al., 2007; Javed et al., 2013; Shadid et al., 2013; Kan et al., 2015).

1.5 Factors influencing primary stability

1.5.1 Implant Morphology and Design

Implant morphology refers to the three-dimensional configuration of an implant with all the different components and features that characterizes it (Javed et al., 2013). Regarding implant design, it can be divided into macro and microdesign. The former includes thread geometry and body shape, while the latter consists of implant material and surface treatment and morphology (Ryu et al., 2014).

1.5.1.1 Length and diameter

There is a huge controversy around this subject. Some studies reported that implant diameter has the greatest influence on implant stability, while implant length showed no adverse impact, others contradict these results (Rabel et al., 2007).

In several studies implant length shows correlation with implant stability (Winter et al., 2010). Sim and Lang conducted a study with 32 healthy patients that received 32 implants. The length of the implants used was either 8 or 10 mm and the

diameter the same in both. The authors observed that baseline implant stability quotient (ISQ) values were 59.8 for the 8 mm implants, while 70.3 for the 10 mm implants. (Sim & Lang, 2009). Other studies showed a positive correlation between implant stability and implant length, but only at low levels of osseointegration. However, a linear increase in implant length led to a nonlinear increase in implant stability (Winter et al., 2010). A study performed by Barikani et al. are in agreement with the aforementioned. They conducted a clinical study where 60 implants with two different lengths (10 mm and 13 mm) and three different widths (3.4 mm, 4.3 mm, and 5mm) were placed into bone blocks with different bone quality. Based on the results of this study, implant length only had influence on PS in cases of insufficient bone quality, in which increasing implant length resulted in an increase of implant PS (Barikani et al., 2013). On the other hand, Ostman et al. reported that not always the use of longer implants resulted in higher PS. They compared PS of implants with the lengths of 7, 8.5, 10, 11.5, 13, 15, and 18 mm. The authors found that by increasing the implant length from 8.5 to 10 mm, PS increases. However, for a range of implant lengths from 10 to 13 mm, PS is almost constant. They also found that implants with 15 and 18 mm resulted in lower PS compared to implants with 13 mm. A possible explanation for this is the higher heat generated due to the longer bone drilling that implants with larger lengths require (Ostman et al., 2006).

Not only greater implant length, but also greater implant diameter has been shown to result in higher resonance frequencies (Winter et al., 2010). Ostman and colleagues, in a study already cited, compared implants of different diameters: 3.75 mm 4 mm, and 5 mm. They found that implants with 5 mm in diameter had significantly higher values of PS, which could be useful in the posterior regions of the jaws. However, differences between 3.75 mm and 4 mm implants weren't observed (Ostman et al., 2006). Furthermore, results from a study conducted by Barikani et al. are in agreement with those (Barikani et al., 2013). In contrast with the aforementioned, Bilhan et al. compared the effect of different implant diameters (3.8 mm versus 4.6 mm) on PS in cancellous bone and found no statistically significant differences in ISQ values (Bilhan et al., 2010). Additionally, some clinical studies shows that implants with 3 or 3.3 mm in diameter could also provide sufficient PS in cases with a limited bone volume (Degidi et al., 2009; Hallman, 2011).

1.5.1.2 Macrodesign

Originally, endosseous implants were produced in a parallel design. However, the original design was not suitable for all applications (Chong et al., 2009; Javed et al., 2013). Tapered (conical) implants were later introduced, among other reasons, to enhance aesthetics (Javed et al., 2013).

In contrast to cylindrical implants, tapered implants allow compressive forces to be distributed to the surrounding bone as they are inserted. The wider coronal portion of the tapered implant will continue to engage the thinner osteotomy site as the implant is inserted deeper (Kan et al., 2015), thereby creating a more uniform compaction of bone in adjacent osteotomy walls compared with parallel implants (Chong et al., 2009). Indeed, implants with a tapered morphology have been shown to achieve more consistent PS than implants with a cylindric morphology (Kan et al., 2015). Also, in cases of limited bone available after extraction, the smaller apical portion of the tapered implants enables better bone engagement with the implants (Kan et al., 2015).

According to the literature, cylindrical implants increase the risk of labial bone perforation, especially in thin alveolar ridges, due to presence of buccal concavities. On the other hand, the decrease in diameter of the tapered implants, toward the apical region, accommodates for the labial concavity (Chong et al., 2009; Javed et al., 2013).

Although several studies reported that no statically significant difference in ISQ value existed between different implant design types (Yoon et al., 2011), other studies demonstrated higher RFA and insertion torque values for tapered implants than for non-tapered implants (Chong et al., 2009). Therefore, in order to enhance PS, must be choosen a tapered implant, which creates lateral bone compression at the moment of implant insertion (Markovic et al., 2011). However, because high compression may result in bone resorption, the degree of taper should be adequate in order to achieve great PS without inducing resorption in the local bone (O'Sullivan et al., 2000).

Other important characteristic of the implant design, for implant PS, is the implant neck configurations. It can be critical for minimizing the marginal bone loss. This area is important because the transition from endosteal environment to oral cavity occurs here. In addition, this is the region where the thick cortical bone is located and the oclusal stress is concentrated (Ryu et al., 2014).

Concerning thread design, it seems to decrease the compression of the crestal bone, thus preventing bone loss (Javed et al., 2013). Thread design should maximize

implant surface area and reduce stress accumulation (Yoon et al., 2011; Javed et al., 2013). Implants with smaller pitch (number of threads per unit length) showed a greater surface area and better stress distribution particularly in low-density bone (Yoon et al., 2011). Regarding thread depth, in high-density bone, shallow threads lead to easier implant placement. On the contrary, deep threads increase the functional surface area at the bone-implant interface, which can improve PS in the low-density bone (Ryu et al., 2014).

1.5.1.3 Microdesign

Many studies have already proved that rough surfaces favor PS by enlarging the implant area in contact with alveolar bone. Also, surface topography and roughness positively affect the healing processes, promoting positive cellular responses. The rate and degree of osseointegration was found to be superior for the rough surface as compared with the machined ones (Tabassum et al., 2009). Indeed, clinical studies have been suggesting that implants with rough surfaces have a failure rate (3.2%) five times lower than machined surface implants (15.2%) (Khang et al., 2001).

Sandblasted and acid-etched are examples of rough implant surfaces. *In vitro* studies (Guizzardi et al., 2004; Franchi et al., 2007) have shown that sandblasted and acid-etched implant surfaces promote peri-implant osteogenesis by enhancing the growth and metabolic activity of osteoblasts, as compared with machined ones.

1.5.2 Surgical Technique

Primarily and regardless the surgical technique used, the surgical trauma should be minimized, in order to prevent injury to the bone, thus preventing bone loss (Digholkar et al., 2015).

Two main surgical techniques have been described as advantageous for improving PS: undersized drilling technique and lateral bone condensation technique. In the conventional bone drilling technique, implant sites were gradually enlarged with pilot and spiral drills using intermittent motions without additional pressure and with a certain drill speed (Markovic et al., 2011).

Undersized drilling technique was introduced to locally optimize the bone density by using a final drill with a significantly smaller diameter compared with the implant diameter (Shadid et al., 2014). This technique increases PS, especially in the

low-density bone (Javed et al., 2013; Shadid et al., 2014; Kan et al., 2015). This can be explained with the improvement of bone-implant contact and preservation of residual cortical bone (Rastelli et al., 2014). A systematic review evaluated whether the undersized drilling technique could enhance the implant PS. No significant difference between the undersized drilling and the standard techniques were observed, but it was clearly in favor of the undersized group (Shadid et al., 2014).

Regarding the bone condensation technique, it consists of first preparing a small-sized pilot hole and then compressing the bone tissue laterally and apically with an implant-shaped instrument (condensers/osteotomes). The aim of this technique is to place the implant with a high degree of stability, without removing additional bone (Javed et al., 2013; Shadid et al., 2014). Several *in vitro* studies have proved that bone condensation technique significantly increases trabecular thickness and bone-to-implant contact. Some authors stated that by condensation technique, peri-implant bone density could be enhanced (Markovic et al., 2011). Two recent studies found positive association between using the osteotome technique and the PS (Shayesteh et al., 2013; Markovic et al., 2013). They demonstrated a statistically significant higher PS for implants placed with osteotome technique than those placed with the conventional drilling technique. Nevertheless, Padmanabhan & Gupta found the opposite results in their study (Padmanabhan & Gupta, 2010). Despite the *in vitro* studies published, there is weak evidence suggesting that the use of this technique in low quality bone could enhance primary and secondary implant stability (Shadid et al., 2014).

Recently, studies have been developed about piezoelectric ultrasonic surgical system. The piezoelectric surgery claims to be superior to conventional methods in several ways: improved precision, selective cutting action, minimal injury to soft tissues, reduced bleeding resulting in improved visibility within the surgical field, and the absence of overheating (Shadid et al., 2014). However, the abovementioned review demonstrated that there was no real difference in PS when piezoelectric technique is used versus the conventional drill technique (Shadid et al., 2014).

All the aforementioned techniques imply a full thickness flap in order to access alveolar bone for implants placement. Yet, with flapless procedure, is possible to place an implant without reflecting any mucoperiosteal flap. Thus, in flapless procedure the bone remains covered by the periosteum. This may increase vascularity of the

peri-implant mucosa. Although, evidence suggesting that flapless procedure could enhance PS is still weak (Shadid et al., 2014).

1.5.3 Bone quality and quantity

A poor bone quantity and quality have been indicated as the main risk factors for implant failure (Markovic et al., 2011; Javed et al., 2013). Bone type was found to affect implant PS, whereas, after healing it exerted only a minor influence (Winter et al., 2010).

According to some authors, failures in implantology are more related to quality than to quantity of bone (Rastelli et al., 2014). Bone quality is defined as the amount (and their topographic relationship) of cortical and cancellous bone in which the implant is placed (Javed et al., 2013). Ostman et al. pointed out that in high bone quality, the greater incidence of cortical bone, which is 10 to 20 times more rigid than cancellous bone, could be the cause of high PS (Ostman et al., 2006). Literature has shown strong correlation between ISQ value and cortical bone thickness, which suggests that cortical bone thickness plays a crucial role for implant PS (Yoon et al., 2011).

Clinical studies have reported higher survival rates of dental implants in the mandible (Javed et al., 2013). In contrast, the highest rate of implant failure reported is in the posterior maxilla, which contains a thin cortical bone combined with thick trabecular bone (Type IV) (Turkyilmaz et al., 2006; Markovic et al., 2011; Javed et al., 2013). It has been assumed that the difference in survival rates of implants placed in maxilla and mandible resulted from the bone conditions around the implants. In the mandible, bone has better volume and quality than in maxilla (Turkyilmaz et al., 2006).

In 1985, Lekholm and Zarb classified bone density radiographically into four types: I - Large homogenous cortical bone; II - Thick cortical layer surrounding a dense medullar bone; III - Thin cortical layer surrounding a dense medullar bone; IV - thin cortical layer surrounding a sparse medullar bone (Lekholm & Zarb, 1985). Currently this bone classification system is still one of the most popular among clinicians. Clinical studies conducted after implant installation have shown that type IV bone has a much higher failure rate when compared with the three other types (Huang et al., 2000).

Other popular method for bone density classification was proposed by Misch in 1990, who defined four bone density groups, regardless of the regions of the jaws, based upon macroscopic cortical and trabecular bone characteristics. Specifically, the density

was defined as D1 if dense cortical bone was present; D2 if thick dense-to-porous cortical bone on crest and coarse trabecular bone was detected; D3 if thin porous cortical bone on crest and fine trabecular bone within was observed; and D4 if fine trabecular bone was present (Misch, 2007). According to a study by Barikani et al., the ISQ values measured for implants placed in D1 bone type are significantly higher than those measured for implants with the same length and diameter, but placed in D3 bone type (Barikani et al., 2013).

With respect to both classifications, more objective and reliable methods are needed to clarify bone characteristics of the surgical sites. Accordingly, Computerized Tomography (CT) has been lately used for evaluation of the bone density of patients requiring implant therapy. Schwarz and colleagues were the first describing the use of CT for this purpose (Schwarz et al., 1987). Indeed, CT is an objective method for evaluating the relative distribution of cortical and cancellous bone, as well as the quantity of bone available (Javed et al., 2013).

1.6 Methods for evaluating primary stability

Implant stability is crucial to satisfactory treatment outcome. Thus, being able to objectively determine it levels will increase implant's success rates (Digholkar et al., 2015). Methods of accessing PS could be either invasive or non-invasive.

1.6.1 Invasive Methods

Assessment of removal torque and histomorphometric evaluation provides reliable data on both strength of bone-implant interface and quality of implant anchorage in peri-implant bone. Though, because of its destructive nature these measures are applicable in an experimental environment only (Park et al., 2011).

1.6.1.1 Removal Torque

Removal torque forces have been used as a biomechanical measure of osseointegration in which greater forces required to remove implants may correspond to an increase in the degree of osseointegration. As a matter of fact, it provides, essentially, information on the rigidity of the bone-implant connection. Therefore, removal torque testing might not be the best test for the evaluation of PS or the amount of bone around the implant (Koh et al., 2009).

In respect to the drawbacks of this technique, measurements of removal torque require destruction of the study specimens and it can only be used after implant placement. Thus, it is impossible to use for long-term assessment (Koh et al., 2009; Markovic et al., 2011).

1.6.1.2 Histomorphometric Analysis

Histomorphometric analysis is obtained by calculating the peri-implant bone quantity and bone-implant contact from a dyed specimen of bone that is removed from the jaws, for instance. A great advantage of this technique is the accurate measurement that it provides. However, due to the invasive and destructive procedure, it is not appropriate for clinical studies (Park et al., 2011b).

1.6.2. Non-invasive Methods

1.6.2.1 Radiographical Evaluation

Radiographical evaluation is a non-invasive method that can be executed at any stage of healing. It has been reported that 1.5 mm of radiographical crestal bone loss can be expected in the first year of loading in a stable implant, with 0.1 mm of subsequent annual bone loss (Mall et al., 2011).

Implant position is an example of important information given by conventional periapical or panoramic views (Digholkar et al., 2015). A relevant drawback of this method is the difficulty of using a standardized technique to ensure good reproducibility (Meredith et al., 1996). Also, it doesn't provide information on a facial bone level, and, as documented in the literature, bone loss at this level precedes mesiodistal bone loss (Mall et al., 2011; Digholkar et al., 2015).

1.6.2.2 Clinical Perception

The clinical perception of implant PS is often based on the mobility detected by blunt ended instruments. It can also be checked by the cutting resistance of implant during its insertion: the feeling of "good" stability may be emphasized if there is the sense of an abrupt stop at the seating of the implant (Mistry et al., 2015).

The drawbacks of this method are numerous: it's a very unreliable and nonobjective method (Mistry et al., 2015), can only be performed when the implant is inserted and is obviously not possible to quantify and to use as a basis for future

comparison (Digholkar et al., 2015).

1.6.2.3 Percussion Test

Percussion test is one of the easiest methods that can be used to estimate the level of osseointegration, and thus PS. This test is based upon vibrational-acoustic science and impact-response theory. The clinical judgment is based on the sound heard after percussion with a metallic instrument: "crystal" (successful osseointegration) "dull" (no osseointegration) (Mall et al., 2011; Mistry et al., 2015).

Due to the poor qualitative information, high subjectivity, and lack reliability of this method (Digholkar et al., 2015; Mistry et al., 2015), it cannot be used experimentally as a standardized testing method (Mall et al., 2011; Mistry et al., 2015).

1.6.2.4 Insertion Torque

The main purpose of insertion torque (IT) is to quantify the initial torque (in Ncm units) required to place the implant into the bone, during surgery, by means of a torque application device and thereby predicts the bone support and density (Shadid et al., 2014). Greater insertion torque means higher density of bone (Rabel et al., 2007).

Regarding the values of IT that are considered adequate, some authors suggest that a minimum of 30 Ncm should be used (Rabel et al., 2007). Others have been chosen 32, 35, or 40 Ncm and higher values as thresholds for immediate loading (Javed et al., 2013). Certain studies reported that for achieving osseointegration, an insertion torque above 32 Ncm is required (Digholkar et al., 2015).

Increased IT has been associated with higher implant survival rates and fewer complications (Kan et al., 2015). Actually, high IT leads to an increase of implant PS (O'Sullivan et al., 2000). However, it may lead also to overcompression and negative tissue effects (Molly, 2006).

Disadvantages of using IT technique for assessing PS include the fact that it only allows a single measurement at implant insertion, it cannot be used for long-term assessment, and cannot be used for evaluating SS. (Markovic et al., 2011; Park et al., 2011; Park et al., 2011b; Shadid et al., 2014; Digholkar et al., 2015). Thus, it cannot collect longitudinal data to assess implant stability changes (Park et al., 2011b).

1.6.2.5 Periotest®

Periotest® (Siemens, Germany) was devised by Dr. Schulte in 1983 (Park et al., 2011b). This electronic instrument was originally designed to measure the damping features of the periodontal ligament and other tissues surrounding a tooth, allowing to obtain a value for its mobility. With similar principles, it has been widely used to measure implant mobility (Rabel et al., 2007; Digholkar et al. 2015). The Periotest® comprises a handpiece containing a metal slug which is accelerated towards an implant by an electromagnet. The contact duration of the slug on the implant is measured by an accelerometer (Meredith et al., 1996). Signals produced by tapping are converted to "Periotest values (PTV)". These values are displayed digitally and audibly on a scale of −8 (low mobility) to +50 (high mobility). The lower the PTV, the higher the implant stability (Shadid et al., 2014). Yet, some authors advocate that there is no absolute PTV that can be regarded as acceptable, being more meaningful the observation of variations of values that occur over time (Mistry et al., 2015).

There are some variables that may influence PTV, such as quality of the hard tissue surrounding the implant, vertical measuring point on the implant abutment, handpiece angulations, and horizontal distance of the handpiece from the implant (Meredith et al., 1996; Rabel et al., 2007; Shadid et al. 2014).

Periotest® can be employed at all stages of treatment: from PS testing, through the healing period, to the finished prosthetic rehabilitation (Digholkar et al., 2015). Aparicio, in an 8-year report had measured PTV at several time intervals after placing implants in 315 patients. An important finding was the correlation of the PTV post-operatively and secondary failure and thus, the possibility of early detection (before fabrication of the dental prosthesis) of a failing implant (Aparicio, 1997).

Lack of resolution, poor sensitivity and susceptibility to operator variables are drawbacks of Periotest® (Rabel et al., 2007; Park et al., 2011; Shadid et al. 2014). Moreover, Periotest® is incapable of detect small changes in the implant-bone interface and barely sensitive to differentiate between osseointegrated and non-osseointegrated implants (Huang et al., 2000; Park et al., 2011b; Herrero-Climent et al., 2013).

1.6.2.6 Resonance Frequency Analysis

In 1994, Meredith et al. described a non-invasive method in which by measuring the resonance frequency of a small transducer attached to an implant fixture and

stimulated by different frequencies, bone formation around an implant could be studied in vivo (Meredith et al., 1996; Javed et al, 2013). Two years later, in 1996, appeared the first studies on resonance frequency analysis as a method for measuring implant stability (Quesada-García et al., 2009). In 1998, studies conducted by Meredith and Sennerby concluded that RFA was a highly effective qualitative method and proposed its use to assess implant stability (Meredith, 1998; Meredith & Sennerby, 1998). In 2002, Huang et al. reached similar conclusions after evaluating implant behavior in different types of bone (Huang et al., 2002).

According to Meredith and colleagues, RFA indicates the stiffness of the transducer-implant-tissue system. Increasing bone anchorage of an implant would alter the resonance characteristics because of changes in stiffness of transducer-implant system in its peri-implant bone (Meredith et al., 1996). Therefore, changes in resonance frequency could indicate changes in the anchorage of the implant (Park et al., 2011).

This technique expresses the implant stability by reading the implant stability quotient, obtained through the RFA. The ISQ values range from 1 to 100 with higher values indicating higher implant stability (Barikani et al., 2013). ISQ values greater than 65 have been associated with high implant PS, while ISQ values below 45 indicate a poor primary stability (Javed et al., 2013).

RFA it's becoming widely used to monitor the changes in stiffness at the implant-tissue interface and to discriminate between successful implants and clinical failures (Shadid et al., 2014). Meredith et al. suggested that, as a baseline reading for future comparison, this test should be performed at implant placement (Meredith et al., 1996). Yet, measurements of PS with RFA can also be made at any time of the healing period and after loading implants with the provisional restoration (Sim & Lang, 2009). That is, RFA could be use for predicting implant success, for selecting the appropriate loading protocol, as well as for monitoring a specific implant during healing and prosthetic reconstruction (Krafft et al., 2015). However, evaluation is impossible in a definitive prosthesis state or when the magnetic peg is damaged or in contact with the soft tissue (Park et al., 2011b; Javed et al., 2013; Mistry et al., 2015).

For assessing PS with RFA method the most used devices are the ones from Osstell® system. While in the first generation of Osstell® the transducer was connected to the instrument through a cable, in the last two models (Osstell® Mentor and Osstell ISQ®, Osstell AB, Gothenburg, Sweden) the transducer, computerized analysis and

excitation source, represents one machine only (Mall et al., 2011; Herrero-Climent et al., 2012; Digholkar et al., 2015). In Osstell® Mentor and Osstell® ISQ the transducer, known as SmartPeg, is screwed on the implant with a force of 5-10 Ncm and communicates with the instruments through electro-magnetic waves (Quesada-García et al., 2009; Herrero-Climent et al., 2012). Both of them are handheld wireless, devices. Additionally, compared with the first generation device, the last two devices are faster and more resistant to electromagnetic noise (Digholkar et al., 2015).

With respect to the technique behind RFA, the manufacturer recommends that the probe (excitation source) be held perpendicular to the alveolar crest for the first measurement and in line with the crest for the other measurement, preserving a distance of about 1-2 mm to the SmartPeg. It must be 3 mm above the soft tissue. When magnetic resonance frequency is released from the probe, the magnetic peg is activated and starts to vibrate. As the SmartPeg attached to the implant vibrates the magnet on its top induces an electric voltage into the probe coil (Park et al., 2011b). The peg vibrates in two directions, which are approximately perpendicular to each other. The vibration takes place in the direction that gives the highest resonance frequency (first mode) and in the direction that gives the lowest resonance frequency (second mode) (Sennerby & Meredith, 2008). The frequency of the registered oscillation varies with the stiffness of the bone-implant attachment: the stiffer the system is, the higher the transducer's oscillation frequency will be (Herrero-Climent et al., 2012).

Due to its high reproducibility and reliability, this technique has progressively outperformed all techniques previously proposed to monitor implant stability (Herrero-Climent et al., 2013). Although a specific transducer has to be used for each implant system, ISQ values obtained can be compared independently of the implant system used (Markovic et al., 2011), which is an important positive aspect of RFA method. Lachmann et al. conducted a study in which they compared reliability of Osstell® and Periotest®. They concluded that Osstell® offers more precise data than Periotest® (Lachmann et al., 2006; Lachmann et al., 2006b).

According to factors influencing RFA measurements, alveolar bone quality appears to be one major parameter (implants placed in mandibular bone shows higher ISQ values than those placed in the maxilla). On the contrary, factors such as length and diameter seems to have only minor effects on ISQ levels (Krafft et al., 2015). Regarding other variables, tightness with which the transducer is attached to the implant (Meredith

et al., 1996) and different positioning of the probe tip appears to influence ISQ values, affecting RFA technique reproducibility (Geckili et al., 2012; Ibáñez M. et al., 2014).

Additionally, several factors related to how the SmartPeg is used can influence RFA method. The manufacturer claims that in order for the SmartPeg to function properly it needs a firm attachment to the implant or abutment, warning also to never use excessive force to screw the SmartPeg in because it could be damage. The SmartPeg is made from soft aluminum, so that it can be much softer than the implant itself, thus reducing the risk of damaging the implant and its connection (Duddeck & Faber, 2015). Nevertheless, the wear and tear of aluminum are fast and visible after only a few inserts. Likewise, the manufacturer states that Osstell instrument will not provide accurate ISQ values if the connection between the SmartPeg and the implant is not perfect ("Why are SmartPegs single use only", 2015).

The manufacturer recommends that SmartPeg is designed for single session use only (Duddeck & Faber, 2015). However, because of the high costs that this kind of utilization will represent for the clinicians, many of them reuse the device after autoclave sterilization.

According to the manufacturer, autoclaving is thought to speed up the wear and tear, but also the corrosion of the aluminum because of the rather extreme conditions during the autoclaving process ("Why are SmartPegs single use only", 2015). In a recent study, was suggested that friction traces on the threads increased dramatically after five or more remounting processes of the SmartPeg. Moreover, aluminum particles may detach after several reuses and remain in the inner part of the implant. However, regarding fatigue fracture test no differences were observed between single used SmartPegs and reused ones. Yet, the authors concluded that reused of the SmartPeg have significant effects on the device and should be avoided (Duddeck & Faber, 2015).

1.7 Clinical Relevance

In sites with high density bone, in which during implant placement, it fails to seat fully, two procedures may be done (1) increase the insertion torque or (2) enlarge the osteotomy site. It is known that large bone compression may result in high rates of bone resorption. However, effects of high insertion torque values on bone are not still clarified. Thus, it seems to be more safe to enlarge the osteotomy site in those types of situations. One of the aims of this study is to evaluate if enlarging the implant site, by

modifying the manufacturer's surgical protocol, the values of implant stability are not altered.

Another aim of the present *in vitro* study is to investigate if the sterilization of the SmartPeg will in fact affect ISQ-readings, thus affecting the assessment of implant PS. Given that (1) the manufacturer states that data integrity cannot be guaranteed after reusing the SmartPeg, only approving the RFA method using Osstell devices if new SmartPegs were used; and (2) the almost absent of studies in the literature that confirms or contradicts this idea. Additionally, because magnetic RFA devices can provide multi-directional measurements and the literature existent is contradictory regarding this subject, it is intended to study if different positioning of the probe tip will result in different ISQ values for the same implant.

2. Aims and Hypothesis

2.1 Aims of the study

Objective 1: To evaluate the influence of two different surgical preparation protocols in the implant primary stability.

Objective 2: To evaluate if different position of the probe tip (perpendicular *vs* parallel) influences the measurement of implant primary stability using RFA technique.

Objective 3: To evaluate if sterilization of the SmartPeg influences primary stability measurements using RFA technique.

2.2 Hypothesis of the study

Hypothesis of the objective 1:

H0: The surgical preparation protocol doesn't influence the implant PS.

H1: The surgical preparation protocol influences the implant PS.

Hypothesis of the objective 2:

H0: The position of the probe tip (perpendicular *vs* parallel) doesn't influence PS measurements.

H1: The position of the probe tip (perpendicular *vs* parallel) influences PS measurements.

Hypothesis of the objective 3:

H0: The sterilization of the SmartPeg doesn't influence PS measurements.

H1: The sterilization of the SmartPeg influences PS measurements.

3. Materials and Methods

3.1 Study Type

In vitro study

3.2 Study Groups

Group A: 10 implants placed according to the manufacturer recommendations and PS measured with the probe tip on a perpendicular direction, using a new SmartPeg.

Group B: 10 implants placed according to the manufacturer recommendations and PS measured with the probe tip on a parallel direction, using a new SmartPeg.

Group C: 10 implants placed according to the manufacturer recommendations and PS measured with the probe tip on a perpendicular direction, using a SmartPeg with 1 cycle of sterilization.

Group D: 10 implants placed according to the manufacturer recommendations and PS measured with the probe tip on a parallel direction, using a SmartPeg with 1 cycle of sterilization.

Group E: 10 implants placed with an altered surgical protocol and PS measured with the probe tip on a perpendicular direction, using a new SmartPeg.

Group F: 10 implants placed with an altered surgical protocol and PS measured with the probe tip on a parallel direction, using a new SmartPeg.

Group G: 10 implants placed with an altered surgical protocol and PS measured with the probe tip on a perpendicular direction, using a SmartPeg with 1 cycle of sterilization.

Group H: 10 implants placed with an altered surgical protocol and PS measured with the probe tip on a parallel direction, using a SmartPeg with 1 cycle of sterilization.

3.3 Sample Size

10 implants were used per group.

3.4 Implants

The implants used in this study were 4.0 x 8.5 mm with an external connection (Implant Double®, Conexão, Rubeaspharma, Porto, Portugal). These are self-tapping implants with parallel walls and a conical apex.

3.5 Bone model

In order to simulate the placement of implants in a similar environment to the human jaw bone, fresh cow ribs were used in this study. It served as a model of human jaw bone because of their cortical and medullar bone composition (Geckili et al., 2012).

3.6 Surgical Perforation Unit

The surgical perforation unit used was the Osseoset 100® (Nobel Biocare, Zurich, Switzerland).

3.7 Surgical Preparation Protocol

3.7.1 Implant site preparation

For groups A, B, C and D the implant site preparation was made according to the manufacturer recommendations, for situations of high density bone - type I/II (Appendix A). The preparation started with a pointed starter drill to identify the implant placement site. After that, the following sequence were used: (1) 2 mm twist drill (nº1); (2) 2.4/2.8 mm twist drill (nº2); (3) 2.8/3.2 mm twist drill (nº5); and (4) 3.4 mm twist drill (nº6). All the drills perforated 8,5 mm in depth.

For groups E, F, G and H (altered preparation protocol) the initial preparation was the same of the manufacturer's protocol groups, but an additional drill were used at the end to enlarge the coronal third of the implant site (3.75/4 mm countersink drill).

Figure1. Perforation with a pointed starter drill

Figure 2. Perforation with a 2 mm twist drill

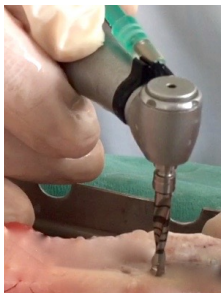


Figure 3. Perforation with a 2.4/2.8 mm twist drill

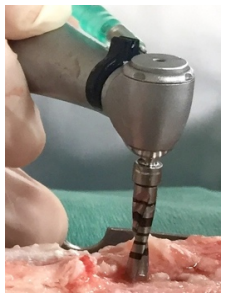


Figure 4. Perforation with a 3.4 mm twist drill

Figure 5. Enlargement of the coronal third with a 3.75/4 mm countersink drill

3.7.2 Implant placement

All implants were placed at the level of the bone crest.

Implants of groups A, B, C and D were placed with a 60 Ncm insertion torque and implants of groups E, F, G and H were placed with a 40 Ncm insertion torque.

3.7.3 Measuring of implant primary stability

Implant PS was measured through RFA obtained with Osstell® Mentor (Osstell AB, Gothenburg, Sweden). The SmartPeg was attached manually without soft-tissue interposition. The probe tip was placed approximately 2 mm from de SmartPeg. In groups A, B, E and F six measurements were taken for each implant, using a new SmartPeg. Three of them were obtained positioning the probe parallel to the SmartPeg and other three positioning it perpendicularly. The final ISQ value was the average of the three measurements. In groups C, D, G and H, also six measurements were taken for each implant, but using a re-used SmartPeg (1 cycle of sterilization). Three of them were obtained positioning the probe parallel to the SmartPeg and other three positioning it perpendicularly. The final ISQ value was the average of the three measurements. Information about the technique behind Osstell® Mentor can be accessed in appendix B.

Figure 6. Probe tip in a perpendicular direction

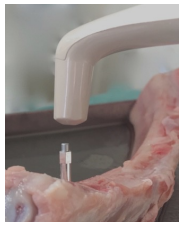


Figure 7. Probe tip in a parallel direction

3.8 Study Variables

3.7.1 Dependent Variable: Primary implant stability measured through resonance frequency analysis with Osstell® Mentor.

3.7.2 Independent Variables: Surgical placement protocol, position of the probe tip, cycles of sterilization of the SmartPeg.

3.9 Statistical Analysis

For statistical analysis of the results the SPSS (Statistical Package for the Social Science v.19, SPSS Inc., Chicago, United States of America) program were used. Kolmogorov-Smirnov and Shapiro-Wilk Tests were used for detecting the suitability of the parameters to normal distribution. Once this hypothesis was rejected, this is, normal distribution wasn't observed, Mann-Whitney Test (nonparametric test) was used in order to compare the different groups by pairs.

The level for statistical significance was set at 5% ($p<0.05$) for all tests performed.

4. Results

The results of the study regarding measurements of PS obtained through Osstell® Mentor are summarized in Appendix C.

A non-parametric test was applied due to the small size of the sample and because after performing **Kolmogorov-Smirnov** it was verified that for all groups the measurements did not follow a normal distribution. Since the intention was to compare the groups by pairs, the nonparametric test **Mann-Whitney Test** was performed.

4.1 Evaluation of the influence of surgical preparation protocol on implant primary stability measured through Osstell® Mentor

The relationship between the values of implant primary stability, obtained through RFA assessed by Osstell® Mentor (expressed in ISQ units), from the implants placed by the manufacturer recommendations or with an altered surgical preparation protocol are shown in Table 1 and Figure 8 (Appendix D).

The results show that **there are significant differences** between the values of implant primary stability of the implants placed in accordance to the manufacturer recommendations and following an altered surgical preparation protocol, concerning measurements with parallel and perpendicular position of the probe tip, and with a new and a re-used (1 cycle of sterilization) SmartPeg ($p < 0.05\%$).

Regarding the groups comparison by pairs, were detected that ISQ of **Group A** (77.47 ± 4.48) presented a higher and statistical significant ($p < 0.05$) mean value than the **Group E** (70.37 ± 3.27). The ISQ of **Group B** (77.67 ± 4.49) presented a higher and statistical significant ($p < 0.05$) mean value than the **Group F** (71.23 ± 3.41), the ISQ of **Group C** (77.57 ± 3.14) presented a higher and statistical significant ($p < 0.05$) mean value than the **Group G** (69.20 ± 5.27) and the ISQ of **Group D** (78.37 ± 3.80) presented a higher and statistical significant ($p < 0.05$) mean value than the **Group H** (69.07 ± 5.28).

It was concluded that implants in Groups A, B, C and D (Manufacturer preparation protocol) presented higher values of implant primary stability than the ones in groups E, F, G and J (Altered preparation protocol).

Table 1

ISQ values according to the surgical preparation protocol

Sterilization cycles and site		**Surgical Preparation Protocol**						
		Manufacturer's Preparation Protocol (MPP)			**Altered Preparation Protocol (APP)**			***p*-Value**
		Mean ± SD	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**	
0	**Perpendicular**	77.47 ± 4.48 (A)	70	85	70.37 ± 3.27 (E)	65	75	0.000
	Parallel	77.67 ± 4.49 (B)	70	85	71.23 ± 3.41 (F)	65	75	0.000
1	**Perpendicular**	77.57 ± 3.14 (C)	70	81	69.20 ± 5.27 (G)	59	78	0.000
	Parallel	78.37 ± 3.80 (D)	70	85	69.07 ± 5.28 (H)	57	79	0.000

4.2 Evaluation of the influence of probe tip position on the ISQ values

Table 2 and Figure 9 (Appendix D) show the comparison between the ISQ values assessed by Osstell® Mentor, from a perpendicular or parallel position of the probe tip.

The results show that **there are no significant differences** between ISQ values assessed by a perpendicular or parallel position of the Osstell® Mentor probe tip, concerning the two different surgical preparation protocols performed and measurements with a new and a re-used (1 cycle of sterilization) SmartPeg ($p > 0.05\%$).

Regarding the groups comparison by pairs, were observed that ISQ of **Group B** (77.67 ± 4.49) presented a higher but not statistical significant ($p > 0.05$) mean value than the **Group A** (77.47 ± 4.48). The ISQ of **Group F** (71.23 ± 3.41) presented a higher but not statistical significant ($p > 0.05$) mean value than the **Group E** (70.37 ± 3.27), the ISQ of **Group D** (78.37 ± 3.80) presented a higher but not statistical significant ($p > 0.05$) mean value than the **Group C** (77.57 ± 3.14) and the ISQ of **Group H** (69.07 ± 5.28) presented a lower but not statistical significant ($p > 0.05$) mean value than the **Group G** (69.20 ± 5.27).

The RFA measurements were higher although not significantly in Groups A, C and E (Parallel position of the probe tip) than the ones in groups B, D and F (Perpendicular position of the probe tip). Also, RFA measurements were lower although not significantly in Group G (Perpendicular position of the probe tip) than the ones in groups H (Parallel position of the probe tip).

Table 2

ISQ values according to the position of the probe tip

Sterilization cycles and surgical protocol		**Site**						
		Perpendicular			**Parallel**			***p*-Value**
		Mean ± SD	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**	
0	**MPP**	77.47 ± 4.48 (A)	70	85	77.67 ± 4.49 (B)	70	85	0.721
	APP	70.37 ± 3.27 (E)	65	75	71.23 ± 3.41 (F)	65	75	0.343
1	**MPP**	77.57 ± 3.14 (C)	70	81	78.37 ± 3.80 (D)	70	85	0.415
	APP	69.20 ± 5.27 (G)	59	78	69.07 ± 5.28 (H)	57	79	0.940

4.3 Evaluation of the influence of sterilization of the SmartPeg on the ISQ values

The relationship between the values of implant PS, obtained through RFA measured with Osstell® Mentor (expressed in ISQ units), using a new SmartPeg or re-used one (1 cycle of sterilization) are shown in Table 3 and Figure 10 (Appendix D).

The results showed that **there are no significant differences** between ISQ-readings obtained through Osstell® Mentor using a new SmartPeg or re-used one (1 cycle of sterilization), concerning the two different surgical preparation protocols performed and measurements with parallel and perpendicular position of the probe tip.

With respect to groups comparison by pairs, were observed that ISQ of **Group B** (77.67 ± 4.49) presented a lower but not statistical significant ($p > 0.05$) mean value than the **Group D** (78.37 ± 3.80). The ISQ of **Group A** (77.47 ± 4.48) presented a lower but not statistical significant ($p > 0.05$) mean value than the **Group C** (77.57 ± 3.14), the ISQ of **Group F** (71.23 ± 3.41) presented a higher but not statistical significant ($p > 0.05$) mean value than the **Group H** (69.07 ± 5.28) and the ISQ of **Group E** (70.37 ± 3.27) presented a lower but not statistical significant ($p > 0.05$) mean value than the **Group G** (69.20 ± 5.27).

Table 3

ISQ values according to the sterilization cycles

Surgical protocol and site		**Sterilization cycles**						
		0			**1**			***p*-Value**
		Mean ± SD	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**	
MPP	**Perpendicular**	77.47 ± 4.48 (A)	70	85	77.57 ± 3.14 (C)	70	81	0.982
	Parallel	77.67 ± 4.49 (B)	70	85	78.37 ± 3.80 (D)	70	85	0.852
APP	**Perpendicular**	70.37 ± 3.27 (E)	65	75	69.20 ± 5.27 (G)	59	78	0.435
	Parallel	71.23 ± 3.41 (F)	65	75	69.07 ± 5.28 (H)	57	79	0.094

5. Discussion

The surgical technique used for implants installation is an extremely important factor to establish a strong bone-to-implant interface, which will determine implant PS (Albrektsson et al., 1981). In fact, surgical trauma and anatomical conditions are thought to be the key factors for early implant loss (Esposito et al., 1998).

The results of the present study suggest that in high density bone, significantly higher values of implant PS are achieved when the standard manufacturer's surgical preparation protocol is performed instead of an altered one, in which the coronal third of the implant site is enlarged with an additional countersink drill. Therefore, the null hypothesis (H0) of the objective 1 (Surgical preparation protocol doesn't influence the implant PS) was rejected with a confidence interval of 95%. The results of this *in vitro* study are in agreement with previous ones, which also concluded that surgical technique influences implant PS (Shalabi et al., 2006; Shayesteh et al., 2013; Markovic et al., 2011). Andrés-García and colleagues performed an *in vitro* study, similar to the current one, also using cow ribs and the same two surgical preparations protocols, and reached the same results (Andrés-García et al., 2008). Additionally, Yoon et al. conducted a study in which implants were placed in high density bone (Type I and II), using three different surgical techniques. The authors concluded that the surgical technique affects the PS of dental implants (Yoon et al., 2000). However, other studies reached the opposite results (Rastelli et al., 2014; Shadid et al., 2014). Shadid et al. stated that there is weak evidence suggesting that any of the five surgical techniques analyzed could influence the PS or SS (Shadid et al., 2014). Handelsman stated that countersinking may cause loss of initial stabilization at time of implant placement, which in fact occurred in the present study (Handelsman, 2006).

In some clinical situations, such as the presence of a high density bone (Type I, with a huge amount of cortical bone) when following the manufacturer recommendations, the implant fails to seat fully. If that happens the clinician could, for instance, increase the IT or enlarge the implant site with a countersink drill. It is largely accepted that an undersized drilled site and high values of IT will result in higher implant PS values. Actually, in the present study, significant higher PS values were obtained when the manufacturer surgical protocol were used (without countersinking). Nevertheless, several studies have also described some negative effects of it on the bone-implant interface, specially in high density bone.

Smaller drilling dimensions might increase PS (as shown in the present study). However, a great amount of remodeling will take place, potentially decreasing implant stability over time. On the other hand, larger drilling dimensions (in this study obtained by a countersink drill) will result in lower implant PS, but also in a lower amount of necrotic bone and remodeling, thanks to the lower levels of compressive strain generated. This might increase the speed of SS achievement (Campos et al., 2012). In fact, the surgical technique also affects the implant SS, which depends on bone modeling and remodeling at the implant-bone interface (Shadid et al., 2014; Digholkar et al., 2015). According to a study performed by Coelho et al., the smaller the drilled site the more pronounced was the amount of remodeling at the interface region. As new bone formation is necessary to replace bone that is remodeled away, this could be a drawback of smaller drilled sites (Coelho et al., 2013). Additionally, Tricio et al. defended that in sites with a cortical thickness of 2 mm or more undersized drilling should be avoided, as over tightening of the implant may lead to micro fractures of the bone threads around the implants (Tricio et al. 1995).

It is described in the literature that the extent of surgically induced bone necrosis at implant installation is mainly due to the frictional heat generated by bone cutting, although additional tissue trauma may be caused by compression of the bone surrounding the implant (Eriksson & Albrektsson, 1984). Concerns have been forwarded that a high IT (>45 Ncm) may lead to overcompression of the bone and negative tissue effects (Molly, 2006; Trisi et al., 2011). Khayat et al. stated that excessive tightening creates important compression forces in the bone (Khayat et al., 2013). High bone compression leads to disturbance of the local microcirculation (ischemia), which may result in necrosis of the osteocytes and bone resorption (O'Sullivan et al., 2000; Trisi et al., 2011). This deleterious effect would be even more pronounced in denser bone (O'Sullivan et al., 2004). Despite the demonstrated positive correlation between high IT values and PS (O'Sullivan et al., 2000; Kan et al., 2015), implant placement with high IT values could lead to concentration of stress on the bone (Barone et al., 2015). Huiskes et al. have shown a direct correlation between high stressed regions and bone resorption (Huiskes et al., 1984). Several studies showed that high IT could in fact had negative effects on the bone, such as micro fractures, resorption in the cortical zone, and a delayed healing process (Barone et al., 2015). Other studies in an animal model have shown that implants with a high IT do not induce

bone necrosis or implant failure (Duyck et al., 2010; Trisi et al., 2011). Still, the association between bone compression and density resulted in a significant marginal bone loss (Duyck et al., 2010). In a clinical study conducted by Khayat et al. the authors concluded that the use of high IT did not have negative effects on osseointegration (Khayat et al., 2013).

Low degrees of bone stress are advantageous, as a lower amount of remodeling would be necessary during osseointegration. Accordingly, during implant insertion minimal pressure should be done to the cortical bone, thus avoiding marginal bone loss and delayed healing. Cortical bone is very dense and consequently has less vascularity, which increases the chance for necrosis, when compressed during implant placement (Bashutski et al., 2009). Barone and colleagues conducted a randomized clinical trial in which they compared clinical outcomes of implants placed with high IT (>50 Ncm) and regular IT (<50 Ncm). They observed that after a year, the overall bone resorption for implants in the regular IT group was 0.69 mm *versus* 1.18 mm in the high IT (Barone et al., 2015). Also, the authors concluded that implants belonging to the high-IT group showed greater marginal bone loss in the mandible than in the maxilla, which could be explain by the greater amount of cortical bone present in the mandible, where the bone compression effects are higher (Barone et al., 2015).

Though no strong consensus has yet been reached on the adequate IT that leads to good implant PS without negatively influencing the bone, several studies suggested a IT value between 30 and 50 Ncm (Rabel et al., 2007; Trisi et al., 2011; Javed et al., 2013). In the present study, implants placed with higher IT values (60 Ncm) reached significant higher values of PS, compared to the ones placed with 40 Ncm. However, the RFA measurements were obtained only at the time of the implant placement, which is a limitation of the current study, since for precisely evaluated the effects of the surgical techniques on bone healing and osseointegration, RFA measurements should be taken over time.

The use of an altered surgical protocol, in which the implant site was enlarged with a countersink drill, was proposed due the described negative effects of high IT values and undersized preparation on sites with high density bone. The countersinking of the implant bed will facilitate seating of implant collar within the cortical bone, specially when dense bone is encountered (Al-Samman & Suleiman, 2012). However, this altered preparation protocol conducted to lower values of PS in all groups tested

(77.77 ISQ for the M.P.P. versus 69.96 ISQ for the A.P.P). Nevertheless, ISQ values greater than 65 have been associated with high PS. Clinical attention should be paid to the risk of high IT, while implant placement, in sites where the cortical bone component is well represented. Further clinical studies with assessing of implant PS over time should be performed to investigate how much compression is advantageous and whether there are any deleterious effects of inducing high compression into the bone.

Given the importance of implant PS on dental implants prognosis, it is extremely important to precisely determine its values during different treatment stages. RFA is considered an easily practicable, accurate, and noninvasive way of determining implant PS (Huang et al., 2000; Rabel et al., 2007; Herrero-Climent et al., 2012; Shadid et al., 2014). This technique can reproducibly assess the bone-to-implant contact (Barikani et al., 2013). Thus, higher ISQ value means that the implant is presumed to be more firmly anchored in the bone, which means more PS (Rabel et al., 2007; Yoon et al., 2011). However, some studies defend that ISQ values at implant placement are not predictive of implants osseointegration (Krafft et al., 2015). Moreover, the relation between RFA and bone structure is not yet clear (Park et al., 2011b). Also, it is stated the sensitivity of RFA may be related to the performance of the equipment used (Markovic et al., 2011), which could be an important drawback of this technique.

Several factors could influence RFA method, such as the distance between the transducer and the first bone contact (Meredith et al., 1996), effective length of the implant above the bone crest, design of the transducer, and stiffness of the implant (Chong et al., 2009; Sennerby & Meredith, 2008). Other factors, directly related to bone conditions, are degree of bone-to-implant contact, thickness of cortical bone, and bone density (Park et al., 2011; Park et al., 2011b). Moreover, positioning of the probe tip and sterilization of the SmartPeg have also been proposed as possible influencing factors.

The results of this study indicate that both position of the probe tip (perpendicular *versus* parallel) and sterilization of the SmartPeg do not influence PS measurements obtained through RFA, since no statistical significant differences were observed between ISQ values recorded with different probe positions and using a new or a 1 cycle of sterilization subjected SmartPeg. Consequently, the null hypothesis (H0) of the objectives 2 (Position of the probe tip doesn't influence primary stability measurements) and 3 (Sterilization of the SmartPeg doesn't influence primary stability

measurements) could not be rejected.

Regarding position of the probe tip, the results of the current study are in agreement with other previous ones (Ohta K. et al., 2010; Park et al., 2011b). Ohta and colleagues conducted a study in pig ribs to evaluate the factors that affect ISQ values using wireless RFA device. The authors concluded that ISQ values were not affected by the direction of the probe from parallel to perpendicular to the long axis of the bone or to the SmartPeg (Ohta K. et al., 2010). Park et al., performed a clinical study in which RFA measurements with Osstell® Mentor were taken twice in each direction (Parallel and Perpendicular) and the mean of the two measurements was regarded as the representative ISQ of that direction. They concluded that probe positioning had no influence on the reproducibility of the ISQ values (Park et al., 2011b).

Nevertheless, other studies suggest that measurements obtained by several clinicians may show variable results owing to different positioning of the probe tip and forces applied during the attachment of the SmartPeg (Tozum et al., 2009; Geckili et al., 2012; Ibáñez et al., 2014). Ibáñez and coworkers (Ibáñez et al., 2014) investigated if the position of the probe perpendicular vs parallel will result in different ISQ values, using Osstell® ISQ. In this *in vivo* study 102 implants were placed and RFA technique was performed to obtain implant PS. For each implant, six measurements were taken, three of them positioning the probe parallel to the SmartPeg and other three positioning it perpendicularly. After a proper statistic analysis of data, results showed statistical significant differences between ISQ values obtained from the two distinct probe orientations used. In 39 out of 102 implants, ISQ values recorded with a parallel probe position were higher; in 38 implants the values were the same for the two positions; and finally in 25 implants, ISQ values recorded from a perpendicular probe position were higher (Ibáñez et al., 2014).

Due to the absence of consensus in the literature regarding the influence of probe positioning in implant stability measurements with RFA devices, future clinical studies are necessary to reach a conclusion. Yet, standardization of device positioning could be important so that values obtained could be more precise and comparable between studies.

Several factors related to how the SmartPeg is used can influence RFA method. For instance, it is essential to ensure proper fixation of the SmartPeg, as even maximum levels of osseointegration cannot be detected if the stiffness of transducer fixation is

insufficient (Winter et al., 2010). Besides that, re-use and sterilization of the SmartPeg are also described as factors influencing ISQ readings. In fact, the manufacturer recommends that the SmartPeg is designed for single use only. Since autoclave sterilization could result in less precise and reliable ISQ data due to corrosion of the aluminum (Sennerby L., 2015). Nevertheless, in the present study no statistical significant differences were observed between ISQ values obtained using a new SmartPeg or a used one (1 cycle of sterilization). This finding isn't in line with the *in vitro* study by Duddeck & Faber, in which they concluded that effects of autoclave sterilization and remounting processes include unequal ISQ values compared to single used SmartPegs. A possible explanation for this contradictory results is the number of sterilization cycles that the SmartPegs underwent (20 *versus* 1). Despite the lack of studies focusing on the effects of SmartPeg sterilization, some studies defended that limiting the use of each SmartPeg could be useful to enhance the accuracy of RFA technique (Kim et al., 2009; Brizuela-Velasco et al., 2015). Further clinical studies investigating the number of sterilization cycles that would induce modifications on RFA measurements accuracy, should be performed.

In addiction to the limitations already referred, the fact that the current study is an *in vitro* study, in which biological factors of the clinical environment are not contemplated, and the reduced size of the sample, constitute important limitations. Moreover, the transducer tightening to the implants was carried out manually, which may lead to the alteration of some results. In order to achieve more reliable data, tightening of the SmartPeg should be standardized.

6. Conclusion

Within the limitations of the current *in vitro* study, the results suggest that in high density bone, the surgical preparation protocol influences the implant primary stability. Additionally, different positions of the probe tip (perpendicular *vs* parallel) and sterilization of the SmartPeg, do not affect primary stability measurements obtained through RFA with Osstell® Mentor.

8. Appendices

APPENDIX A
Surgical preparation protocol

SURGICAL PROTOCOL

Maxilla - Density III and IV

HE Grip / Easy/Double	Ø 3.3	1+**(2)**	
	Ø 3.75	1+2+**(5)**	
	Ø 4	1+2+5+**(6)**	
	Ø 5	1+2+5+7+**(8)**	
MI Flash	Ø 3.5	1+2+**(5)***	
	Ø 4.3	1+2+5+7*	
	Ø5	1+2+7+8*	
MI AR/Torq/Exp	Ø 3.5	1+2+**(B)**	
	Ø 3.75	1+2+**(5)**+C	
	Ø 4	1+2+5+**(6)**+C	
	Ø 5	1+2+7+**(8)**+D	

Mandible - Density I and II

HE Easy / Double	Ø 3.3	1+2+**(B)**+**(M)**	
	Ø 3.75	1+2+3+4+**(A)**+**(M)**	
	Ø 4	1+2+5+6+**(A)**+**(M)**	
	Ø 5	1+2+7+8+**(D)**+**(M)**	
HE Short	Ø 4,3	1+2+5+7+**(M)**	
	Ø 5,0	1+2+5+7+8+**(M)**	
MI AR/Torq/Exp	Ø 3.5	1+2+B+**(M)**	
	Ø 3.75	1+2+3+4+C+**(M)**	
	Ø 4	1+2+5+6+C+**(M)**	
	Ø 5	1+2+7+8+D+**(M)**	

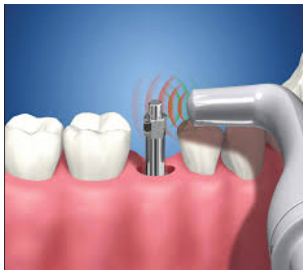
DOUBLE INDICATION

MAXILLA AND MANDIBLE

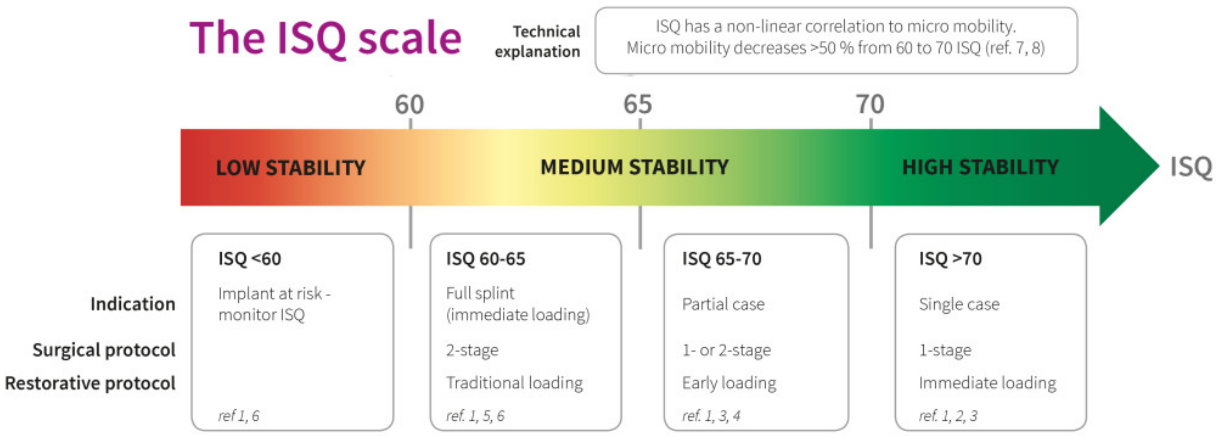
APPENDIX B
The technique behind Osstell® Mentor

1 – A sensor (SmartPeg) is mounted on top of the implant

2 – The probe tip of Osstell device emits magnetic pulses that brought to vibration the SmartPeg



3 – The frequency with which the SmartPeg vibrates is expressed in ISQ (Implant Stability Quotient) units. The ISQ-scale runs from 1 to 100, with higher values meaning higher stability.



APPENDIX C
Tables of ISQ values of implants per group

A - Manufacturer Protocol; Perpendicular direction; New SmartPeg				
	ISQ1	ISQ2	ISQ3	Mean ISQ
1	78	78	78	78
2	82	82	82	82
3	75	75	75	75
4	76	76	76	76
5	78	79	79	78.67
6	84	85	85	84.67
7	81	81	81	81
8	78	78	79	78.33
9	70	70	70	70
10	71	71	71	71
B - Manufacturer Protocol; Parallel direction; New SmartPeg				
1	79	79	79	79
2	79	80	82	80.33
3	74	74	75	74.33
4	80	77	77	78
5	79	79	79	79
6	85	85	85	85
7	81	81	81	81
8	79	79	79	79
9	70	70	70	70
10	71	71	71	71
C - Manufacturer Protocol; Perpendicular direction; SmartPeg with 1 cycle of sterilization				
1	69	69	69	69
2	73	73	73	73
3	68	69	69	68.67
4	72	72	72	72
5	68	68	68	68
6	71	71	71	71
7	75	75	75	75
8	75	75	75	75
9	65	65	65	65
10	67	67	67	67
D - Manufacturer Protocol; Parallel direction; SmartPeg with 1 cycle of sterilization				
1	74	74	69	72.33
2	73	73	73	73
3	70	70	70	70
4	71	72	72	71.67
5	68	68	68	68
6	75	74	75	74.67
7	75	75	75	75
8	75	75	75	75
9	67	65	65	65.67
10	67	67	67	67
E - Altered Protocol; Perpendicular direction; New SmartPeg				
1	69	69	69	69
2	73	73	73	73
3	68	69	69	68.67
4	72	72	72	72
5	68	68	68	68

6	71	71	71	71
7	75	75	75	75
8	75	75	75	75
9	65	65	65	65
10	67	67	67	67
F - Altered Protocol; Parallel direction; New SmartPeg				
1	74	74	69	72.33
2	73	73	73	73
3	70	70	70	70
4	71	72	72	71.67
5	68	68	68	68
6	75	74	75	74.67
7	75	75	75	75
8	75	75	75	75
9	67	65	65	65.67
10	67	67	67	67
G – Altered Protocol; Perpendicular direction; SmartPeg with 1 cycle of sterilization				
1	73	73	73	73
2	59	59	60	59.33
3	77	78	78	77.67
4	73	73	74	73.33
5	71	71	71	71
6	67	67	67	67
7	71	71	71	71
8	71	71	71	71
9	66	66	66	66
10	63	62	63	62.67
H – Altered Protocol; Parallel direction; SmartPeg with 1 cycle of sterilization				
1	73	73	73	73
2	57	60	57	58
3	78	78	79	78.33
4	73	73	74	73.33
5	68	68	68	68
6	67	67	67	67
7	71	71	71	71
8	72	71	71	71.33
9	68	68	68	68
10	62	63	63	62.67

APPENDIX D
Graphics of ISQ values

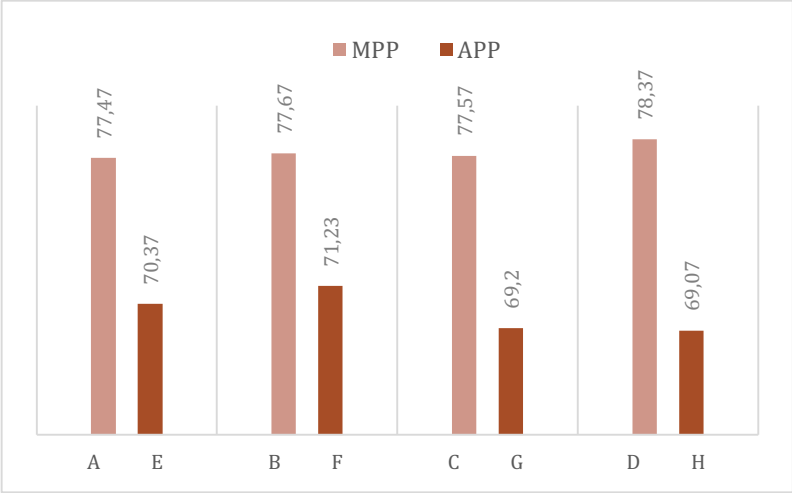


Figure 8. Graphic of ISQ values according to the surgical preparation protocol per group

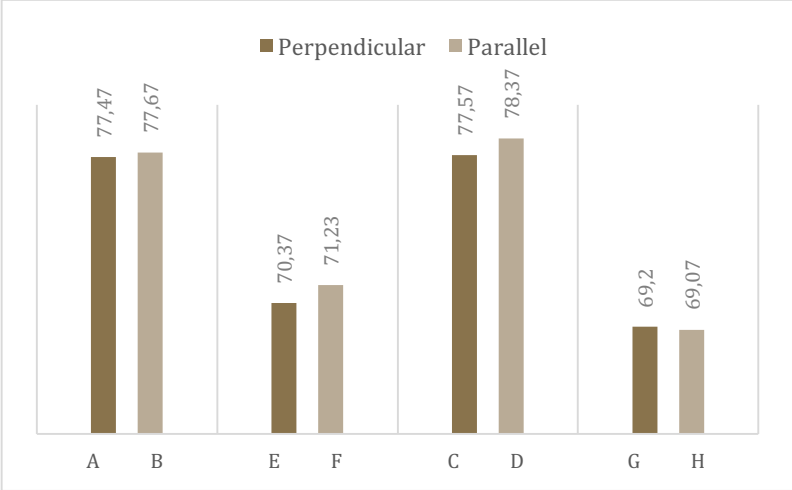


Figure 9. Graphic of ISQ values according to the probe tip position per group

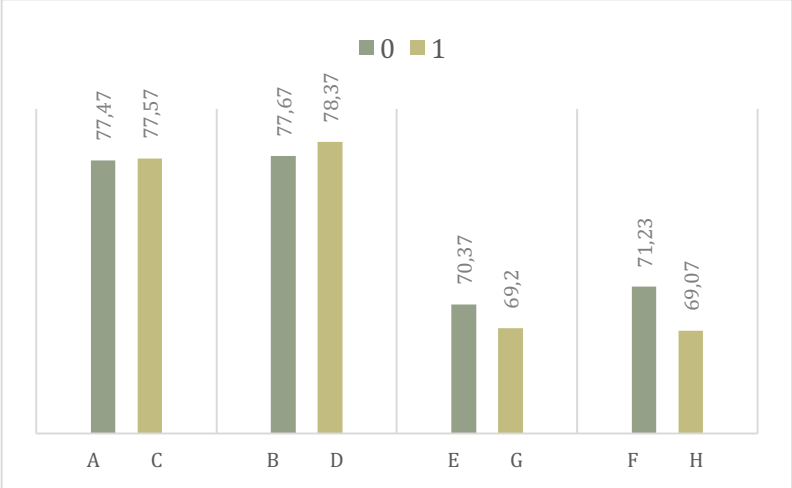


Figure 10. Graphic of ISQ values according to the sterilization cycles per group

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